TEMPERATURE EFFECTS IN REPRODUCTION OF THE BAY SCALLOP, AEQUIPECTEN IRRADIANS LAMARCK[1]

A. N. SASTRY

Duke University Marine Laboratory, Beaufort, North Carolina

The environmental temperature has been considered to be the most important factor in regulating the breeding period in marine invertebrates (Orton, 1920; Runnström, 1927). Korringa (1957) directed attention to variation in temperature requirements of populations of a species from different geographic localities. There is considerable discussion as to how the temperature controls the reproductive activities of marine invertebrates, and recent reviews by Giese (1959) and Boolootian (1964) include relevant literature on this subject. Breeding condition was induced in the oyster, *Crassostrea virginica* Gmelin, during winter by gradually raising water temperature and providing food (Loosanoff and Davis, 1952). The oocytes of the scallop, *Pecten yessoensis,* were accelerated to maturity *in vitro,* by exposure to higher temperatures, long before the population in nature reached maturity (Yamamoto, 1951). Arctic barnacles, *Balanus balanoides* L. and *Balanus balanus* (L.) were reported to require maintenance at 3°–10° C. for one to three months before they could be brought to a breeding condition, while the population in nature was not breeding (Crisp, 1957). Tropical species of barnacles were brought to maturity in winter by maintaining them at higher temperatures and providing food (Patel and Crisp, 1960). Maturation and spawning was advanced in the bay scallop, *Aequipecten irradians* Lamarck, by exposing the animals to higher temperature during winter, while spawning in the natural population normally begins in August (Sastry, 1963). Although these studies indicate that temperature affects the reproductive activities of marine invertebrates, it is not clear to what extent it influences the events in the reproductive cycle or its exact role in determining the annual reproductive pattern. The present study was undertaken to determine the effect of temperature on the events in a reproductive period of the bay scallop, *A. irradians* Lamarck.

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Materials and Methods

Throughout this study, the bay scallops, *A. irradians* Lamarck, were collected from the same grass flat in the vicinity of the Duke University Marine Laboratory,

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Beaufort, North Carolina. The local population has an annual reproductive cycle and individuals commence spawning at the age of one year (Gutsell, 1930). The life span of the species is about two years throughout its range of distribution on the Atlantic and Gulf Coasts of the United States (Belding, 1910; Gutsell, 1930; Sastry, 1961). The animals in the second year are not found in large numbers, either because of commercial fishing at the end of the spawning period or due to natural mortality. Therefore, all the animals used in this study belonged to a year class and were collected from the beginning of their first reproductive period.

Analysis of field animals

After each collection, the animals were brought into the laboratory and gonad index (gonad weight/body weight × 100) and digestive gland index (digestive gland weight/body weight × 100) were determined for a sample of ten animals (Giese, 1959). The digestive gland and the gonad were easily separated from the rest of the body. The bay scallops are functional hermaphrodites, and testis and ovary occupy the proximal and distal regions of the same gonad mass. The gonad condition of each animal in the sample was assayed by microscopical examination of freshly made smears of testicular and ovarian tissues. The oocytes in the ovary were measured with an ocular micrometer in several microscopical fields, and the stage of their development was noted. The gonads were preserved for future histological study.

Laboratory procedure

In some collections, the remaining animals were divided into groups of equal size and maintained at various temperatures covering the annual temperature range characteristic of this region. The gonad coloration of each animal was examined before introducing them to the experimental temperatures. The animals were placed in battery jars containing 7 liters of sea water and maintained at different temperatures. The sea water in the containers was continuously aerated. Initially, the temperature of sea water in the battery jars was allowed to reach the desired experimental temperature over a period of time. On the alternate days, the animals were changed to fresh sea water of equilibrated temperatures. The photoperiod was regulated to provide 12 hours of light and 12 hours of darkness. The salinity of sea water varied between 25‰ and 35‰ during these experiments. No food was supplied to the animals during the entire period of this study.

Temperature effects were assessed by using the following criteria: spawning of animals at the temperature at which they were maintained; changes in gonad coloration; 50% survival of experimental animals at the respective maintenance temperatures was considered as completion of the experiment. At the end of the experiment, the remaining animals were removed and the gonad and digestive gland indices and the microscopical condition of the gonad were determined as described for the field sample. When gametes were released during the experimental period, they were fertilized and development to the veliger stage was followed. Gonads removed from the experimental animals were also preserved for future histological study. The same procedure was followed whenever the animals were maintained

at different temperatures in the laboratory from the collections made at intervals during the reproductive period.

Observations on Field Animals

Changes in gonad and digestive gland indices

The mean gonad and digestive gland indices obtained for the sample analyzed at each collection during the reproductive period are shown in Figure 1. The gonad index rapidly increased during May to early July by about three times. The gonad index remained about the same from early July to early September. Towards the later part of September, the gonad index was about four times greater

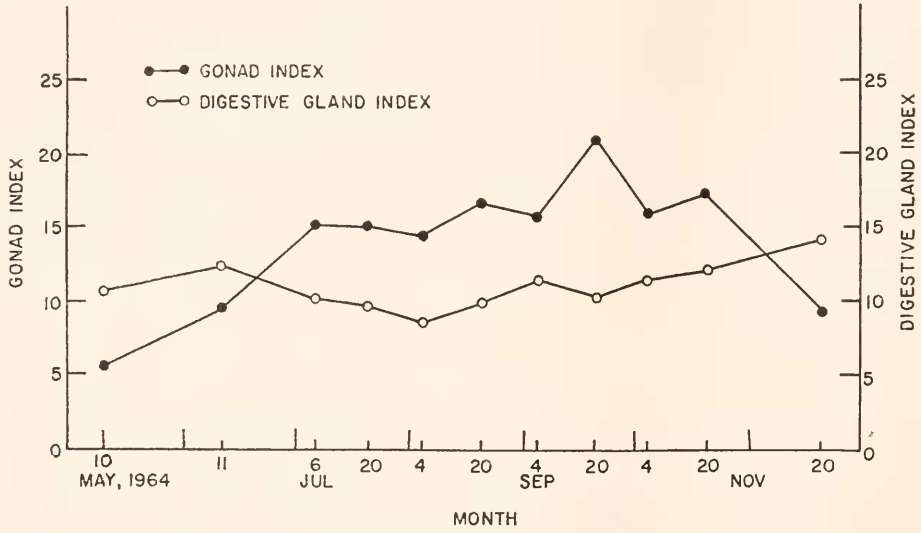

Figure 1. Changes in mean gonad index and digestive gland index of scallops during reproductive period. Note the reciprocal relationship between gonad index and the digestive gland index.

than in May. The gonads of marine invertebrates increase in weight by accumulation of various biochemical reserves when they reach a mature condition (Giese, 1959). A decreasing trend followed the maximum observed in the later part of September and reached a lower value by late November. The spawning period covered approximately two months during September and November. The slight variation observed in the gonad index values in each sample during gonad growth suggests that some individuals might be slower in gathering food or accumulating reserves in the gonads. A similar variation in the gonad index values during spawning period indicates that all the animals in a population might not liberate gametes at the same time, or that they liberate different amounts at any single discharge (Table I).

The digestive gland index was higher than the gonad index in May and early June. The digestive gland index decreased to a value less than gonad index by

TABLE I

Changes in gonad index, digestive gland index and the microscopical condition of gonads in A. irradians during the reproductive period

Date collected	Size mm. mean ±S.D.	Average weight g.	Average body weight g.	Gonad index mean ±S.D.	Digestive gland index mean ±S.D.	Gonad condition		
						Average oocyte diameter μ	Color	Microscopical
5/10/64	33.84 ±4.49	11.33	3.39	5.5 ±1.1	10.75 ±1.72	8.0; range 5.2–13.0	dark	spermatocytes, oogonia, few early oocytes
6/11/64	39.69 ±3.31	16.86	5.52	9.6 ±3.2	12.47 ±1.29	13.0; range 7.8–15.0	dark	sperm, oogonia, oocytes
7/6/64	51.14 ±4.71	32.64	12.28	15.1 ±1.84	10.13 ±1.14	20.3; range 10.4 —45.0	cream, light orange	sperm, oocytes in germinal vesicle stage
7/20/64	56.33 ±4.16	37.69	16.53	15.1 ±1.86	9.80 ±1.21	45.5; range 20.8–72.8	cream, light orange	sperm, oocytes in germinal vesicle stage
8/4/64	56.20 ±4.20	43.19	17.24	14.6 ±1.30	8.50 ±1.00	53.4; range 20.8–72.8	cream, orange	sperm, oocytes with orange pigmentation
8/20/64	59.36 ±2.09	49.99	21.01	16.66 ±4.41	9.93 ±1.10	59.5; range 20.8–91.0	cream, orange	sperm, oocytes with germinal vesicle shrinkage
9/4/64	61.39 ±3.74	52.85	22.02	15.52 ±2.6	11.41 ±0.9	58.5; range 26.0–104.0	cream, orange	sperm, oocytes with germinal vesicle breakdown, few eggs
9/20/64	64.85 ±3.36	63.29	26.95	20.95 ±2.9	10.26 ±1.40	67.6; range 20.8–104.0	cream, bright orange	sperm, eggs, oocytes with germinal vesicle breakdown
10/4/64	65.39 ±1.39	64.77	23.08	15.9 ±2.9	11.46 ±1.47	49.38; range 20.8–98.8	cream, pale orange	sperm, few eggs, oocytes in germinal vesicle stage
10/20/64	63.00 ±2.98	54.25	17.31	17.31 ±1.8	12.05 ±1.08	50.31; range 18.2–104.0	cream, pale orange	sperm, few eggs, oocytes with germinal vesicle breakdown
11/20/64	64.75 ±4.09	63.04	26.22	9.46 ±2.5	14.2 ±0.85	—	pale brown	residual sperm, neutral ovary

the middle of June and a reciprocal relationship was maintained until the end of the reproductive period. An increasing trend in the digestive gland index was observed beginning from the spawning period and it reached a higher value than gonad index by the later part of November.

Changes in gonad coloration

The changes in gonad coloration during reproductive period are as described previously for this species (Sastry, 1963). The entire gonad was covered with a dark peripheral membrane at the beginning of reproductive period. As the gonads begin to differentiate into proximal testicular and distal ovarian regions, a demarcating line separated the two portions. The dark membrane was lost and a cream color in the testis and orange color in the ovaries appeared by the time the gonads reached maturity. The coloration of gonads was gradually lost with the onset of spawning and they were pale brown in spent animals.

Changes in microscopical condition of gonads

The microscopical changes observed during the reproductive period, from May through November, are listed in Table I. The gross microscopical changes of gonads in this species closely follow the descriptions made for other hermaphroditic pectens, by Dakin (1909), Mason (1958) and Reddiah (1962), from detailed

histological studies. In this study, the progressive stages in oocyte development, from primary germ cell to the stage prior to spawning, are described to facilitate understanding of temperature effects to be considered in the following sections of this article.

In May, the testicular region contained a large number of spermatogonia and spermatocytes. From the middle of June until the later part of September, spermatozoa predominated and they were closely packed in the follicles. The testicular region increased with further gonadal growth during this period. Towards the later part of November, only a few spermatozoa were retained.

The process of oocyte development, from primary germ cell to the stage prior to spawning, is similar in many respects to other bivalve molluscs (Raven, 1961). Following the vegetative phase, the gonads contained large amounts of connective tissue and narrow tubules ramified to form indistinct follicles in the ovary. The primary germ cells on the germinal epithelium of the gonad wall develop into oogonia and oocytes (Raven, 1958). The oogonia increased in size, with their free margins protruding into the lumen of the follicles while they were still attached with a stalk to the germinal epithelium. The oocytes detached from the germinal epithelium and began growth by an initial increase in size of the nucleus and nucleolus.

The oocytes in the early stage of development contained a large germinal vesicle surrounded by a thin layer of cytoplasm. The oocytes passed through a rapid growth phase by a simultaneous increase in nuclear and cytoplasmic regions. The cytoplasmic granules were transparent in spherical oocytes of this stage. Following the synchronous growth phase, orange pigmentation appeared in the cytoplasm. Beginning with the appearance of orange pigmentation, the cytoplasmic region increased while the germinal vesicle remained about the same size. Maturation process commenced with an initial shrinkage and later disappearance of the germinal vesicle. Immediately after germinal vesicle breakdown, the pigmented cytoplasmic granules were uniformly distributed in the entire oocyte. The oocytes were of maximum size at this stage (80–104 μ diameter). The diameter of the oocytes decreased and the cytoplasmic granules were closely packed, leaving a clear space between the egg surface and the outer membrane (jelly hull, Costello *et. al.*, 1957). The eggs (average diameter 63 μ) released in this stage completed maturation on fertilization, external to the parent animal.

The changes in average oocyte size during the reproductive period are shown in Figure 2. Few oogonia were present in May. By the middle of June, a large number of oogonia and few oocytes were observed. The oocytes were in the beginning of synchronous growth phase (average diameter, 20 μ) by early July. Towards the later part of July, the oocytes were about 40–45 μ in diameter and some of them began to show orange pigmentation. The pigmented oocytes predominated in early August and germinal vesicle shrinkage was noted by the later part of the month. In early September, few eggs were observed and their number increased towards the end of this month. Although the reproductive condition of each collection was fairly uniform, there was variation in size and exact stage of oocytes in different follicles of the same gonad. While eggs predominated in late September, earlier stage oocytes were also present. Animals examined in

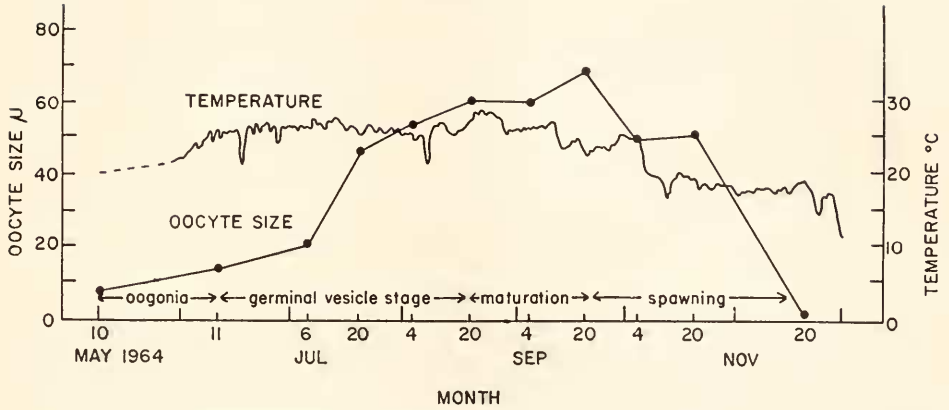

FIGURE 2. Changes in average oocyte size of scallops collected at intervals during the reproductive period. Reproductive condition of the population is shown on the bottom of the graph. The solid line shows the daily noon-time sea water temperature observed in the vicinity where scallops have been collected.

October showed few eggs and oocytes. The gonads decreased in size by the later part of November and the ovaries were empty (Fig. 2).

Changes in environmental temperature during the reproductive period

The daily noon-time water temperatures observed in the vicinity where scallops were collected are shown in Figure 2. The temperature began to increase from about 20° C. in early May to 25° C. by the middle of June. The temperature remained above 25° C. from the middle of June to the middle of September, except for occasional fluctuations. From the middle of September, the temperature began to decrease and it varied between 20° and 25° C. until the beginning of October. The temperature was below 20° C. in early October, and it fluctuated around 17°–18° C. until the later part of November.

Availability of food in scallop habitat

The bay scallops feed on microflora consisting of benthic and tychopelagic diatoms, detritus and bacteria, by filtering the suspended food in the water (Davis and Marshall, 1961). In the shallow estuarine habitat of scallops, the tidal currents continuously stir the sea water and keep the phytoplankton and detritus in suspension. Williams and Murdoch (in press) estimated the phytoplankton production for the same area where scallops have been collected and found the highest production in summer and early fall, and low values throughout the rest of the year (Fig. 3). The phytoplankton production had a pronounced seasonal cycle closely following the temperature cycle. The phytoplankton was found to be chiefly nannoplankton (Williams and Murdoch, in press).

The bay scallops have a relatively high rate of water filtration, probably correlated with their active mode of life and rapid growth rate (Chipman and Hopkins, 1954). The rapid growth of scallops and their gonad development

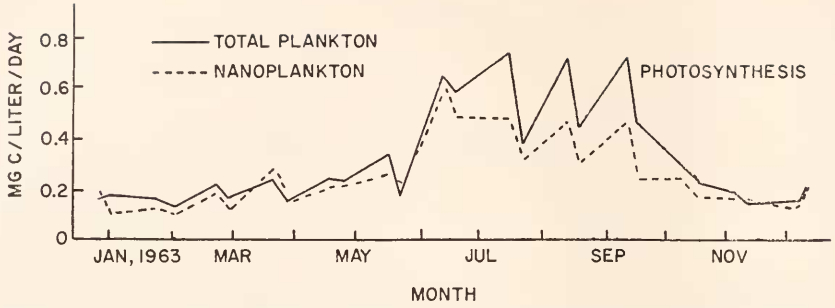

FIGURE 3. The phytoplankton production in the Beaufort Channel, North Carolina. The scallops were collected in the same locality. (The results on phytoplankton production are being published by Dr. Richard Williams, Radiobiological Laboratory, U. S. Fish and Wildlife Service, in a separate paper. I am indebted to Dr. Williams for permitting me to quote his results here.)

(Table I) appear to follow closely the increasing temperature and phytoplankton production beginning in May.

EXPERIMENTAL RESULTS

The groups of animals maintained at various temperatures are shown in Table II. The gonad index, digestive gland index and the microscopical condition of the sample analyzed from the same collection to which these temperature groups belonged, provided the basis for assessing the temperature effects in reproductive period (Table I).

Temperature effects on gonad growth and gametogenesis (Group I)

Temperature effects on group I are shown in Table III. The gonad index and digestive gland index decreased at all the temperatures, but their decrease was more rapid at higher temperatures. The gonads of animals at 10° C. were whitish and spermatocytes were observed in biopsies from testis and ovary. Spermatocytes were observed in gonadal biopsies of animals at 20° C. and 30° C. Oogonia and oocytes were absent. At these temperatures the gonads were translucent, indicating

TABLE II

Groups of scallops collected at intervals during the reproductive period and maintained at different temperatures

Group	Date collected and introduced to experimental temperatures	Experimental temperatures ± 0.5–1.0° C.	Total number of animals	Size mm. mean ±S.D.
I	6/11/64	10, 20, 30	24	35.13 ± 3.28
II	7/6/64	10, 15, 20, 25, 30	50	52.28 ± 3.21
III	8/20/64	10, 15, 20, 25, 30	50	59.24 ± 3.92
IV	10/20/64	10, 20, 25, 30	24	62.06 ± 2.62
V	11/20/64	10, 15, 20, 25, 30	30	65.13 ± 3.55

TABLE III

Temperature effects on scallops during gonad growth and gametogenesis (Group I)

Temperature ° C.	No. animals	Average size mm.	Average weight g.	At the time of 50% survival					
				Average gonad index	Average digestive gland index	Spawned	Gonad condition		
							Average oocyte diameter μ	Color	Microscopical
10	8	34.5	12.81	3.3	10.64	—	absorbed	whitish	spermatocytes
20	8	35.6	14.12	3.2	4.96	—	absorbed	pale brown	spermatocytes
30	8	35.2	13.50	3.2	3.70	—	absorbed	pale brown	spermatocytes

that the gonadal tissue was utilized for maintenance of the animals. The spermatocytes, predominant at 10° C., were reduced in number at higher temperatures.

Temperature effects on animals with accumulated gonad reserves (Group II)

The gonad index of the sample analyzed before introducing this group to experimental temperatures was three times greater than in May and the oocytes were in the beginning of growth phase (Table I). Temperature effects on this group are shown in Table IV. There was a slight decrease in gonad index and

TABLE IV

Temperature effects on scallops with accumulated gonad reserves and oocytes in the beginning of growth phase (Group II)

Temperature ° C.	No. animals	Average size mm.	Average weight g.	At the time of 50% survival					
				Average gonad index	Average digestive gland index	Spawned	Gonad condition		
							Average oocyte μ	Color	Microscopical
10	10	53.4	51.6	14.2	9.3	—	23.0	cream, pale orange	sperm, oocytes
15	10	53.0	41.6	11.1	5.1	—	absorbed	pale brown	spermatocytes
20	10	53.1	38.0	6.0	9.1	—	absorbed	pale brown	spermatocytes
25	10	48.6	34.0	6.9	7.8	8th day sperm, eggs	—	pale brown	residual sperm
30	10	53.3	39.6	10.3	8.1	5th day sperm, eggs	—	pale brown	residual sperm

digestive gland index at 10° C.; however, the oocytes remained in the beginning of growth phase (average diameter, 23 μ). Spermatozoa were retained in the testicular region. The gonad and digestive gland indices decreased at 15° C. and 20° C. On examination of gonadal biopsies, absence of oogonia and oocytes and presence of spermatocytes and spermatozoa were observed.

Eggs and spermatozoa were released at 25° C. and 30° C. on the eighth and fifth day, respectively. On fertilization, the eggs developed to normal larvae, being maintained at the temperature they were released. Apparently, the decrease in gonad index of these animals was partially due to loss of gametes from the gonads.

Effect of temperature on animals with oocytes in the beginning of maturation (Group III)

Table V shows the temperature effects on group III. The temperature effects were similar to those observed for the previous group with two notable exceptions. In addition to the animals at 25° C. and 30° C., those maintained at 20° C. also released gametes. The time for release of gametes in this group was less than that for the previous group (Fig. 4). The gonad index values and observation of gonadal biopsies indicated that they had completely discharged the gametes.

At 10° C. there was no appreciable change in either gonad index or digestive gland index, but the oocytes were observed to disintegrate by rupture of the

TABLE V

Temperature effects on scallops with accumulated gonad reserves and oocytes in the beginning of maturation (Group III)

Temperature °C.	No. animals	Average size mm.	Average weight g.	At the time of 50% survival					
				Average gonad index	Average digestive gland index	Spawned	Gonad condition		
							Average oocyte diameter μ	Color	Microscopical
10	10	58.49	50.85	16.7	8.9	—	57.2	whitish pale orange	mature sperm
15	10	58.64	53.36	14.54	10.24	—	—	cream, pale orange	mature sperm, eggs with ruptured membrane
20	10	59.31	54.46	7.73	9.64	10th day sperm, eggs	—	pale brown	residual sperm
25	10	59.68	56.46	8.20	9.8	6th day sperm, eggs	—	pale brown	residual sperm
30	10	60.08	57.41	9.81	8.4	4th day sperm, eggs	—	pale brown	residual sperm

surrounding membrane. Oocytes in earlier stages of development remained in about the same condition. Spermatozoa were observed in large numbers in the peripheral tissue of the ovary. At 15° C. spermatozoa and disintegrating oocytes were observed in the gonads. The gonad index changed very slightly. Loosanoff and Davis (1951) observed reabsorption of ova, when spawning was delayed by maintaining them at low temperatures.

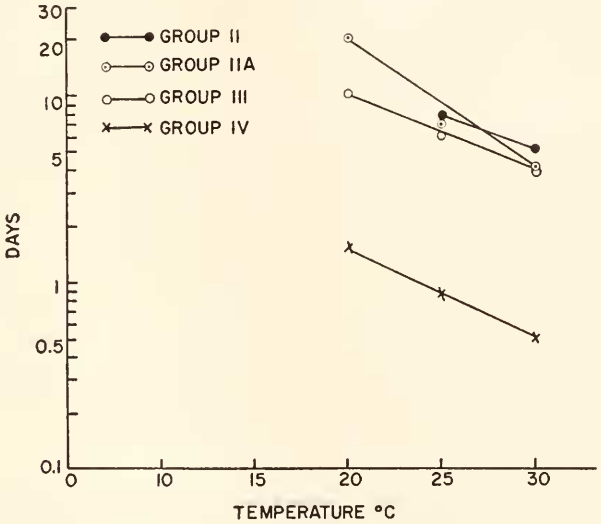

FIGURE 4. The time taken for spawning of scallops at different temperatures in the laboratory. Each group represents a collection made at intervals during the reproductive period. Group IIA is a collection made on July 20, 1964. Note the time-temperature relationship for spawning in different groups.

During September, when the animals were in mature physiological state, they readily liberated gametes even before the sea water in the containers reached the desired experimental temperature.

Temperature effects on animals with partially spent gonads (Group IV)

This group was collected after spawning had commenced in the field population. The results, shown in Table VI, indicate that the temperature effects were similar to those observed for the previous group. The gametes were released at 20°, 25°, and 30° C. The time taken for spawning at these temperatures was less than that for the previous group (Fig. 4). Residual spermatozoa and a few early-stage oocytes were observed on examination of gonadal biopsies. On fertilization, the eggs developed to normal larvae at the temperature they have been released. The animals at 10° C. remained in the same condition as when introduced.

Effect of temperature on animals with spent gonads (Group V)

Table VII shows the temperature effects on group V. The digestive gland index of animals at 10° C. and 15° C. decreased slightly, but the gonad index

TABLE VI

Effect of temperature on scallops with gonads in spawning condition (Group IV)

Temperature ° C.	No. animals	Average size mm.	Average weight g.	At the time of 50% survival					
				Average gonad index	Average digestive gland index	Spawned	Gonad condition		
							Average oocyte diameter μ	Color	Microscopical
10	6	62.4	60.89	20.1	12.35	—	51.06	cream, orange	sperm, oocytes and eggs
20	6	62.2	58.46	10.24	13.32	about 36 hours sperm, eggs	—	pale brown	residual sperm
25	6	62.2	63.32	13.42	11.81	12–30 hours sperm, eggs	—	pale brown	residual sperm
30	6	61.4	59.86	14.40	10.64	about 12 hours sperm, eggs	—	pale brown	residual sperm

remained approximately the same. Residual spermatozoa and a large number of spermatocytes were observed in the testicular region, whereas the ovarian region remained neutral without formation of follicles or oogonia. At 20° C., the gonad index decreased but the digestive gland index remained about the same.

TABLE VII

Temperature effects on scallops with neutral gonads (Group V)

Temperature ° C.	No. animals	Average size mm.	Average weight g.	At the time of 50% survival					
				Average gonad index	Average digestive gland index	Spawned	Gonad condition		
							Average oocyte diameter μ	Color	Microscopical
10	6	65.5	69.39	8.57	12.57	—	—	pale brown	spermatocytes
15	6	64.0	64.52	9.17	11.00	—	—	pale brown	spermatocytes
20	6	64.5	67.26	6.52	11.58	—	—	pale brown	spermatocytes
25	6	65.7	65.96	5.58	6.30	—	—	pale brown	spermatocytes
30	6	66.1	69.29	4.92	8.45	—	—	pale brown	spermatocytes

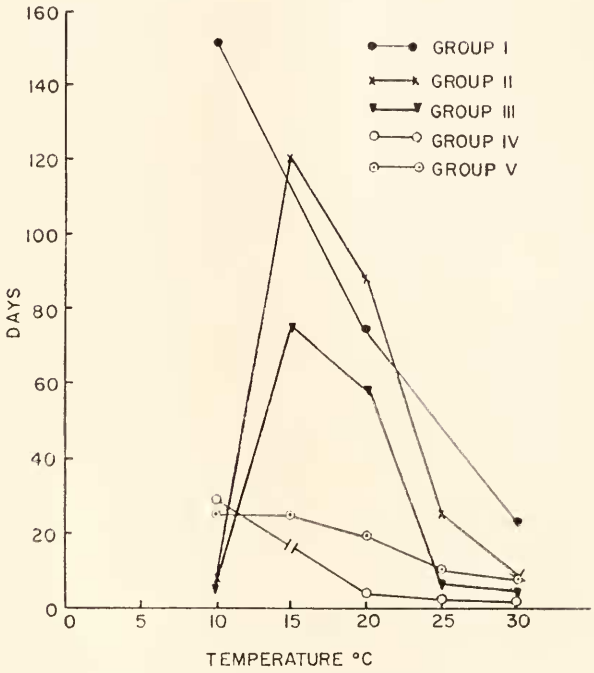

FIGURE 5. The time for 50% survival of scallops maintained at different temperatures. Each group represents a collection made at intervals during the reproductive period.

Some animals at this temperature formed indistinct follicles and a few oogonia. The digestive gland and gonad indices of the animals at 25° C. and 30° C. decreased and no gametogenesis was initiated.

Temperature tolerance of scallops during reproductive period

The time for 50% survival of different groups maintained at various temperatures is shown in Figure 5. Group I tolerated all the temperatures longer than any other group maintained under similar conditions. The tolerance of scallops to 10° C. decreased considerably when the oocytes were developing. Animals in spawning condition were least tolerant to all the temperatures, but those maintained at 20° C. and above released gametes before death. The animals with neutral gonads survived slightly longer than the spawning animals. Temperatures between 15° C. and 25° C. seem to be better suited for scallops in reproductive condition.

DISCUSSION

The reproductive cycles of marine invertebrates include several events: activation of gonad growth following the vegetative phase, gametogenesis, maturation, spawning and the resting stage (Giese, 1959). The suggestion that temperature regulates breeding period (Orton, 1920; Runnström, 1927) and the possible genetic control of differences in breeding temperatures of latitudinally separated populations of a species (Korringa, 1957) are based on only one event in the

reproductive cycle. The results of this study show that temperature influences the events in the reproductive period beginning with gonad growth.

Temperature effects on gonad growth

The scallops exposed to various temperatures during the period of gonad growth (group I), without food supply, showed a decrease in both gonad and digestive gland indices. Although the digestive gland index was greater than the gonad index before this group was introduced to the experimental temperatures, no gonad development was observed at any of the temperatures. This shows that additional food supply is essential for gonad growth and that the reserves accumulated in the digestive gland are not sufficient to support this process. The review of Giese (1959) cites some examples of marine invertebrates in which gonad tissue has been utilized when they were starved long before the breeding period. Barnes, Barnes and Finlayson (1963) point out that food is stored in the boreo-arctic species of barnacles when it is most abundant and utilized for gonad development later in the season, thus ensuring the availability of food during the period of gonad development. It is possible that different species might have adapted differently for nutrient storage and utilization in gonad development.

In the scallops beginning their first reproductive period, growth and gonad development take place simultaneously (Table I), indicating that the ingested food might be rapidly digested and utilized. The mechanism of nutrient transfer to gonads has been considered to be either through blood circulation or direct transfer (Barnes, Barnes and Finlayson, 1963; Lawrence, Lawrence and Giese, 1965). A reciprocal relationship between the gonad index and the digestive gland index during the reproductive period has been reported in *Pisaster ochraceus* (Farmanfarmaian *et al.*, 1958) and *Haliotis cracherodii* (Boolootian *et al.*, 1962). The reciprocal relationship between the gonad and the digestive gland indices of scallops during the reproductive period indicates that the nutrients taken up by the animals might be rapidly utilized for growth and development, without prior storage. It is likely that all the nutrients taken up by the animals might be rapidly utilized and hence a low digestive gland index might result during the reproductive period. The digestive gland index of scallops in the resting stage was higher than the gonad index and this period coincides with decreased environmental temperature and reproductive inactivity. The gonads did not develop when the resting stage animals (immediately after completion of spawning in the field) were maintained at various temperatures without food supply. The gonad index of animals maintained at lower temperature remained the same, whereas the digestive gland and gonad indices decreased at higher temperatures. It appears that all the reserves available in the animals have been utilized for maintenance at higher temperatures. Therefore, it is possible that gonad growth might take place in the presence of abundant food and under temperature conditions at which nutrient mobilization to gonads is permitted after the metabolic needs of the animals have been met.

Temperature effects on gametogenesis, maturation and spawning

Although gametogenesis is initiated when the gonads have a minimum amount of reserves (*i.e.*, May and June; Table I), a continued transfer of nutrient

reserves to the gonads appears to be necessary for further growth and development of gametes (Table III). Various biochemical reserves are mobilized to the gonad synthetic centers when gametes are formed (Giese, 1959; Barnes, Barnes and Finlayson, 1963). Starvation of scallops during the period of gonad growth resulted in an absorption of oogonia and oocytes at all the temperatures. The temperature effects on spermatogenesis were not so pronounced. Spermatocytes were present in the testis and the peripheral tissue of the ovary of animals maintained at all temperatures but they failed to mature (Tables III and VII).

The animals with accumulated gonad reserves (Tables IV–VI) when maintained at 20° C. and above released gametes, even though they were starved. This shows that once a certain amount of reserves is present in the gonads and if the temperatures are suitable for gamete growth to maturity and spawning, these processes might be irreversible. The development of eggs seems to proceed normally without making the reserves available for maintenance of the starving animals. The time at which food is withheld might be critical in gonad growth and gamete development.

When the oocytes are in the beginning of growth phase (Table IV), the animals maintained at 20° C. and below failed to mature and spawn. However, when the oocytes are in the later stages of development, the animals maintained at 20° C. also spawned, but those held at 15° C. and 10° C. failed to spawn. The scallops seem to require temperatures slightly above 20° C. for development of oocytes to the stage prior to release. The temperatures at which spawning occurred decreased the further the oocytes had developed toward maturity. The spawning of the laboratory-maintained animals was ahead of those in the field (Fig. 4, Groups II and III). Crisp (1957) reported that starvation accelerates maturation of barnacles, provided the eggs are developing in the ovary.

The time-temperature relation for spawning of scallops from different collections (Fig. 4) indicates that the development of oocytes to maturity might be a function of temperature within a range characteristic for the species, if the gonads have already accumulated sufficient reserves to support the synthetic activities of the developing gametes. Loosanoff and Davis (1952) showed that the time required for maturation of oysters, *Crassostrea virginica,* is dependent on the maintenance temperature. They also found that oysters with poor glycogen reserves failed to reach maturity. The later stages in gonad development of scallops (*i.e.,* oocyte growth, maturation and spawning) might be influenced by maintaining the animals at optimum temperatures, but the success of these processes appears to depend on the presence of reserves in the gonads. Hence, any factors affecting food collection, or nutrient mobilization to gonads would also affect the reproductive activities of the field population.

Ecological considerations

The reproductive period of the scallops is regulated to the warm temperatures of summer and early fall. The laboratory results on temperature effects on reproduction suggest that the warm temperatures of summer are necessary for oocytes to develop to the stage prior to release. The gonad growth is timed to a period when phytoplankton is most abundant in the environment and this seems to provide enough food for the animals to accumulate reserves in the body for

maintenance and for mobilization to the gonads. The abundance of food has been generally associated with breeding period of marine invertebrates and thought to ensure adequate nutritional availability for the planktonotrophic larvae (see Thorson, 1950; Giese, 1959; Boolootian, 1964, for discussion), but the factors responsible for correlating these events are not clear. Although synchronization of spawning with the period when food is most abundant for larvae is important, the adults also seem to require large amounts of food for gonad growth. A decrease in phytoplankton production during peak spawning period could be seen in Figure 3. The food required for larvae and the adults might be different in their quality and quantity. Sastry (1965) reared the scallop larvae to preadults by providing unicellular algae as food. Additional experiments to determine the value of different organisms in plankton as food for larvae are needed for an understanding of the relationship between food and reproduction.

In general, on the days when spawning occurred in the laboratory animals, it was observed to take place when they were changed to fresh sea water of equilibrated temperatures. Although care was taken, slight changes in temperature and disturbance of animals could not be avoided. It is likely that once the animals have reached a mature physiological state, a slight change in any of the responsible factors might induce a spawning rhythm. Sastry (1963) reported stimulation of spawning in scallops by temperature changes and indicated that spawning might be dependent on the maturation condition of gonads and a favorable stimulus. The lower temperature limits for early cleavages of fertilized scallop eggs have been found to be between 15° C. and 20° C. (unpublished data). The timing of spawning to the period when temperatures are suitable for completion of early stages in development would also be an important factor in regulating the breeding period. Runnström (1936) determined the temperature limits for egg development in a number of marine invertebrates, and Thorson (1950) pointed out that the temperatures during breeding period were within the lethal limits for development of eggs and larvae of a species that can spawn within a given area.

In conclusion, the events in the reproductive period of scallops appear to have been regulated to the period when phytoplankton is most abundant and temperatures are favorable for gonad development. The duration of these two factors in the environment might determine the length of gonad activity. Further experiments are needed to understand the phasing of these events in the reproductive period and the factors responsible for timing of breeding period in nature. Barnes (1963) demonstrated the interaction of light and temperature in gonad development and maturation of barnacles. Boolootian (1963) reported that the testes of *Strongylocentrotus purpuratus* responded differently by production of gonia cells at long-day stimulus, and spermatocytes, spermatids and spermatozoa at reduced day-length. The influence of light and temperature and their mutual interaction on different events in reproduction should be carefully analyzed to determine environmental control of annual reproductive pattern of marine invertebrates.

Summary

1. The events in the reproductive period of a population of bay scallops, *Aequipecten irradians,* have been analyzed from the beginning of gonad growth to the resting or neutral stage. The gonad index of field animals increased rapidly

between May and early July, and the population reached a physiological state to commence spawning towards the later part of September. The spawning period covered approximately two months between September and November.

2. Gonad growth coincides with increasing temperature and peak phytoplankton production in early summer. The gonad index was maximum when the population was ready to commence spawning. The gonad index and the digestive gland index showed a reciprocal relationship during the reproductive period.

3. Scallops collected at intervals during the reproductive period were maintained at various temperatures in the laboratory, without food. The temperature effects on the events in the reproductive period have been studied.

4. Maintenance of scallops at various temperatures during the period of gonad growth resulted in a decrease in digestive gland and gonad indices. The oogonia and the oocytes were absorbed. The spermatocytes in the testis failed to mature.

5. Scallops with accumulated gonad reserves and developing oocytes in the ovary, when maintained at 20°, 25° and 30° C. rapidly matured and spawned. The scallops maintained at 10° C. and 15° C. failed to mature and spawn.

6. When the gonads were neutral, maintenance of animals at any of the experimental temperatures did not permit increase in gonad index or initiation of oogenesis. These animals had a high digestive gland index before they were introduced to the experimental temperatures.

7. The scallops with developing oocytes in the ovary, when maintained at 20°, 25° and 30° C. in the laboratory, released gametes much earlier than the field population. The time required for release of gametes showed a direct relationship to temperature between 20° C. and 30° C.

8. The scallops maintained at various temperatures in the beginning of gonadal growth and gametogenesis survived longer than any other group maintained under similar conditions during the reproductive period. The animals in spawning condition were least tolerant to all the temperatures.

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